CLAIMS

What is claimed is:

1. A method of identifying an agent that modulates microtubule depolymerization, said method comprising the steps of:

(i) contacting a polymerized microtubule with a microtubule severing protein or a microtubule depolymerizing protein in the presence of an ATP or a GTP and said agent; and

(ii) detecting the formation of tubulin monomers, dimers or oligomers, wherein the formation of said tubulin monomers, dimers, or oligomers indicates that said agent modulates microtubule depolymerization.

2. The method of claim 1, wherein said polymerized microtubule is labeled with DAPI.

3. The method of claim 1, wherein said detecting is by fluorescent resonance energy transfer (FRET).

4. The method of claim 2, wherein said detecting comprising detecting a change in fluorescence of said labeled microtubule.

5. The method of claim 1, wherein said detecting comprises centrifuging said tubulin monomers if present.

6. The method of claim 1, wherein said microtubules are stabilized by contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue, and a non-hydrolyzable nucleotide GTP analogue.

7. The method of claim 1, wherein said microtubule is attached to a solid surface.

8. The method of claim 7, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivited microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), and a polylysine..

9. The method of claim 1, wherein said a microtubule severing protein or a microtubule depolymerizing protein is selected from the group consisting of a katanin, a p60 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

10. The method of claim 9, wherein said microtubule severing protein is a katanin or a p60 subunit of a katanin.

11. The method of claim 10, wherein said p60 subunit of a katanin is a polypeptide of claim 26.

12. The method of claim 10, wherein said p60 subunit is a polypeptide having the amino acid sequence of SEQ ID NO: 1.

13. The method of claim 1, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures. each reaction mixture comprising a distinct and distinguishable domain of said array, and wherein said steps are performed in each reaction mixture.

14. The method of claim 13, wherein said array comprises a microtitre plate.

15. The method of claim 13, wherein said array comprises at least 48 of said reaction mixtures.

16. The method of claim 13, wherein said agent is one of a plurality of agents and wherein each reaction mixture comprises one agent of said plurality of agents.

17. A method of identifying a therapeutic lead compound that modulates depolymerization or severing of a microtubule system, said method comprising the steps of:

i) providing an assay mixture comprising a katanin p60 subunit and a microtubules;

ii) contacting said assay mixture with a test compound to be screened for the ability to inhibit or enhance the microtubule severing or ATPase activity of said p60 subunit; and

iii) detecting specific binding of said test compound to said p60 subunit or a change in the ATPase activity of said p60 subunit.

18. The method of claim 17, wherein said detecting comprises detecting ATPase activity utilizes malachite green as a detection reagent.

19. The method of claim 17, wherein said p60 subunit is labeled and said test agent is attached to a solid support.

20. The method of claim 17, wherein said test agent is labeled and said p60 subunit is attached to a solid support.

21. The method of claim 17, wherein said microtubules are stabilized by contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue, and a non-hydrolyzable nucleotide GTP analogue

22. The method of claim 17, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures. each reaction mixture comprising a distinct and distinguishable domain of said array, and wherein said steps are performed in each reaction mixture.

23. The method of claim 22, wherein said array comprises a microtitre plate.

24. The method of claim 22, wherein said array comprises at least 48 of said reaction mixtures.

25. The method of claim 22, wherein said agent one of a plurality of agents and wherein each reaction mixture comprises one agent of said plurality of agents

26. A polypeptide having microtubule severing activity, said polypeptide comprising an isolated p60 subunit of a katanin, wherein said p60 subunit is encoded by a nucleic acid that hybridizes under stringent conditions with a nucleic acid that encodes the amino acid SEQ ID NO: 1.

27. The polypeptide of claim 26. wherein said polypeptide is the polypeptide of SEQ ID NO: 1 or the polypeptide of SEQ ID NO: 1 having conservative substitutions.

28. The polypeptide of claim 26, wherein said polypeptide comprising at least 8 contiguous amino acids from a polypeptide sequence encoded by a nucleic acid as set forth in SEQ ID NO: 1, wherein:

said polypeptide, when presented as an antigen, elicits the production of an antibody that specifically binds to a polypeptide sequence encoded by a nucleic acid as set forth in SEQ ID NO: 1; and

said polypeptide does not bind to antisera raised against a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1, that has been fully immunosorbed with a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1.

29. The polypeptide of claim 26, wherein said polypeptide is the polypeptide of SEQ ID No: 1.

30. An isolated nucleic acid that encodes a katanin p60 subunit having microtubule severing activity, said nucleic acid comprising a nucleic acid that specifically hybridizes with a nucleic acid that encodes the polypeptide of SEQ ID NO:1 under stringent conditions.

31. The nucleic acid of claim 30, wherein said nucleic acid encodes a polypeptide of SEQ ID No: 1 or conservative substitutions thereof.

32. The nucleic acid of claim 30, further comprising a promoter.

33. The nucleic acid of claim 32, wherein said promoter is a baculovirus promoter.

34. A kit for screening for agents that modulate microtubule depolymerization, said kit comprising one or more containers containing an isolated microtubule severing protein or a microtubule depolymerizing protein.

35. The kit of claim 34, further comprising a polymerized microtubule labeled with DAPI.

36. The kit of claim 34, wherein said microtubule is stabilized by contact with paclitaxel or a paclitaxel derivative.

37. The kit of claim 36, wherein said microtubule is attached to a solid surface.

38. The kit of claim 37, wherein said microtubule is attached to said surface by binding with a motor protein.

39. The kit of claim 34, wherein said microtubule severing protein or microtubule depolymerizing protein is selected from the group consisting of a katanin, a p60 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

40. The kit of claim 39, wherein said microtubule severing protein is a katanin or a p60 subunit of a katanin.

41. The kit of claim 34, wherein said p60 subunit of a katanin is a polypeptide of claim 26.

42. The kit of claim 34, wherein said microtubule severing protein or microtubule depolymerizing protein is attached to a solid surface.

43. A method of screening for an agent that alters microtubule polymerization or depolymerization or severing, said method comprising:

providing labeled tubulin;

contacting said labeled tubulin with said agent to produce contacted tubulin;

comparing the fluorescence intensity or pattern of said contacted tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with said agent wherein a difference in fluorescence pattern or intensity between the contacted and the not contacted tubulin indicates that said agent alters microtubule polymerization or depolymerization.

44. The method of claim 43, wherein said labeled tubulin is in the form of tubulin monomers, tubulin dimers, or tubulin oligomers.

45. The method of claim 43, wherein said labeled tubulin is in the form of a microtubule.

46. The method of claim 45, wherein said microtubule is attached to a solid surface.

47. The method of claim 45, wherein said label is selected from the group consisting of DAPI, ANS, Bis-ANS, ruthenium red, cresol violet, and DCVJ.

48. The method of claim 47, wherein said label is DAPI.

49. The method of claim 46, wherein said microtubule is attached to said surface by binding with an agent selected from the group consisting of an inactivated microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

50. The method of claim 43, wherein said contacting further comprises contacting said tubulin with a microtubule depolymerizing protein or a microtubule severing protein.

51. The method of claim 50, wherein said a microtubule severing protein or a microtubule depolymerizing protein is selected from the group consisting of a katanin, a p60 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

52. The method of claim 51, wherein said microtubule severing protein is a katanin or a p60 subunit of a katanin.

53. The method of claim 52, wherein said p60 subunit of a katanin is a polypeptide of claim 26.

54. The method of claim 52, wherein said p60 subunit is a polypeptide having the amino acid sequence of SEQ ID NO: 1.

55. The method of claim 43, wherein said method is performed in an array where said array comprises a multiplicity of reaction mixtures. each reaction mixture comprising a distinct and distinguishable domain of said array, and wherein said steps are performed in each reaction mixture.

56. The method of claim 55, wherein said array comprises a microtitre plate.

57. The method of claim 55, wherein said array comprises at least 48 of said reaction mixtures.

58. The method of claim 55, wherein said agent one of a plurality of agents and wherein each reaction mixture comprises one agent of said plurality of agents.

59. The method of claim 43, further comprising listing the agents that alters microtubule polymerization, depolymerization, or severing into a database of therapeutic lead compounds that act on the cytoskeletal system..